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16. ABSTRACT

Adult male Long-Evans rats were intubated with either 0,5,6,or 7 mg/kg trimethyltin chloride. Activity was measured for 1 hr in a figure-eight maze 2 hr after dosing (day 0) and again on days 4,8,16 and 32 after dosing. On days 49-51, activity was measured in a figure-eight maze over a 23-hr period. There were no differences in activity on the day of dosing, but on all subsequent test days the 7 mg/kg TMT animals were hyperactive. TMT also altered the spatial pattern of activity: activity was increased in the "figure-eight" portion of the maze but not in the blind alleys. Activity of the 7 mg/kg TMT animals was increased during all periods in the 23-hr test. Decreases in the length of the pyramidal cell line (CA1 to CA3c of the hippocampus) confirmed neuronal cell loss in TMT-dosed rats.

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Trimethyltin-Induced Hyperactivity: Time Course and Pattern[1,2]

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Trimethyltin Hyperactivity Hippocampal damage Behavioral toxicology

TRIMETHYLTIN (TMT), a neurotoxic organotin compound, produces a selective pattern of neuronal damage most evident in the hippocampus, although some alterations are also observed in the pyriform cortex, amygdala and neocortex [2]. Limited neuropathological changes are evident as early as 2 days following a single dose of TMT and increase in severity over time with maximal damage seen 21 days following dosing [2]. Within the hippocampus, loss of pyramidal cells primarily in CA4 and CA3 is a conspicuous feature of TMT toxicity [2,6], although the earliest ultrastructural changes may appear in the dentate granule cells [1]. Due to this relatively selective neurotoxicity, TMT may be useful for investigating hippocampal physiology and function.

A commonly reported behavioral change following damage to the hippocampal complex is hyperactivity [9, 14, 21]. Since systemically administered TMT damages the hippocampus, it is likely that this neurotoxicant also affects motor activity. Swartzwelder *et al.* [25] have recently reported that TMT-dosed rats were hyperactive in a 2-min test of open field activity 40 days following dosing. The purpose of the present study was to delineate the time course, dose-dependence and circadian distribution of hyperactivity in TMT-dosed rats. In addition, the length of the pyramidal cell line of the hippocampus was measured as an index of hippocampal damage.

METHOD

Male Long-Evans hooded rats (Charles River), 60 days of age at the start of the study, were housed individually in cages measuring 21×23×21 cm. Animals were allowed free access to both Purina Lab Chow and water, and were weighed daily throughout testing. The animal room was maintained on a 12 hr light/dark cycle beginning at 0600 hr; behavioral testing was conducted during the diurnal period, except where noted.

On day 0, rats (N=10/group) were intubated with either 0 (physiological saline), 5, 6 or 7 mg/kg trimethyltin chloride (ICN, Plainview, NY) measured as the base and dissolved in saline in a volume of 1.0 ml/kg. Time-dependent changes in locomotor activity were determined during 1 hr test sessions in a figure-eight maze 2 hr after dosing, and again 4, 8, 16, and 32 days after dosing. To determine whether TMT altered the circadian pattern of activity, each animal was tested for 23 hr activity in the maze on days 49–51 following dosing. The maze (Fig. 1) consists of a series of interconnected alleys (10×10 cm) converging on a central open arena, and covered with transparent acrylic plastic [22]. Motor activity was detected by 8 phototransistor/photodiode pairs; a count is registered each time a light beam is interrupted. A total of sixteen mazes were housed in two sound attenuated rooms maintained on a photoperiod identical to the animal quarters. Each rat was tested individually in a maze.

For assessment of hippocampal damage, 10 μm paraffin sections cut in the sagittal plane were stained with cresyl violet. Sections were projected onto plain white paper for viewing (1 mm=37 μm). Nine slides were prepared from sequential sections beginning at 2 mm from the midline, and samples for measurement were obtained from alternate slides. The length of the pyramidal cell line was measured to the nearest mm from the CA1 subiculum border until the

[1]This paper has been reviewed by the Health Effects Research Laboratory, U. S. Enviornmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.
[2]Presented at Society for Neuroscience Annual Meeting, Los Angeles, CA, 1981.

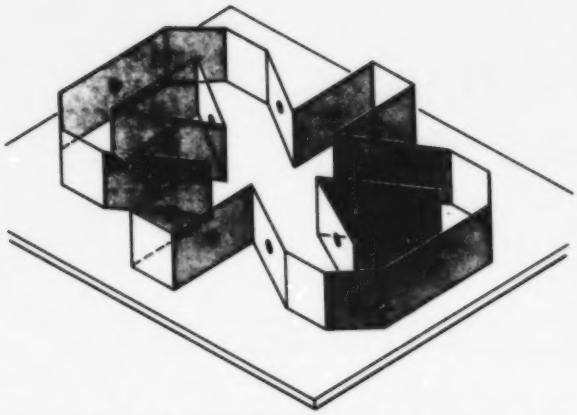

FIG. 1. Figure-eight maze. The location of photocells (N=8) which detect motor activity is indicated by dark cirles.

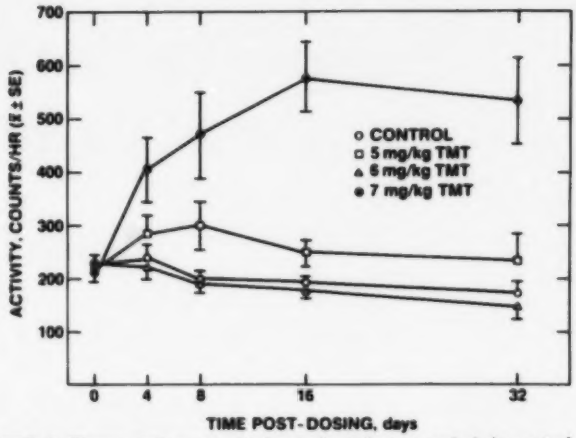

FIG. 2. Figure-eight maze activity during 1-hr-test periods in control and TMT-dosed rats (N=9–10/group). Values are mean ± SE for total photocell counts at each time post-dosing.

organized line of pyramidal cells was no longer easily discriminated (CA3c in normal tissue). Further details of the method can be found in Dyer *et al*. [6].

Body weight, total 1 hr maze activity, distribution of photocell interruptions and rate of habituation (R) within the hour were analyzed by a two factor analysis of variance for effects of treatment and day of testing. The rate of habituation (R) was defined by the equation: activity = $Ie^{-(R)(TIME)}$, where I represents initial activity. Non-linear regression [5] was used to estimate I and R for each animal. Total activity for the 23-hr test and length of the pyramidal cell line were analyzed for a treatment effect by a one factor analysis of variance. Post-hoc comparisons were made using Tukey's (a) test [27].

RESULTS

Within days of dosing, animals treated with 7 mg/kg TMT exhibited hyperexcitability and spontaneous seizures. These behavioral effects and their time course have been described previously [7]. Signs were first evident 5–10 days following dosing and disappeared by post-exposure day 16. One animal in the 7 mg/kg group died 8 days after dosing; data from this animal were not included in the statistical analysis. At no time during testing did body weights significantly differ between groups.

Within 4 days of dosing, animals treated with 7 mg/kg TMT exhibited hyperactivity which persisted throughout testing (Fig. 2). There was a significant effect of treatment, $F(3,140)=16.00$, $p<0.0001$, day, $F(4,140)=3.66$, $p<0.0072$, and a treatment-by-day interaction, $F(12,140)=7.08$, $p<0.0001$. On day 0, there were no differences between groups in maze activity. However, on days 4, 8, 16, and 32 there were differences in activity, $F(3,38)=4.42$, 8.34, 29.87, and 13.34, respectively, $p<0.0098$. In all cases, the 7 mg/kg TMT animals were more active than controls ($p<0.01$); the 7 mg/kg TMT animals were more active than 6 mg/kg animals on days 4–32 ($p<0.01$), and more active than 5 mg/kg animals on days 8–32 ($p<0.05$). The slight but non-significant increase in activity in 5 mg/kg group seen in Fig. 2 was due to one hyperactive animal in this group.

The distribution of photocell interruptions is shown in Fig. 3. The maze was divided into 3 areas (see Fig. 1): the

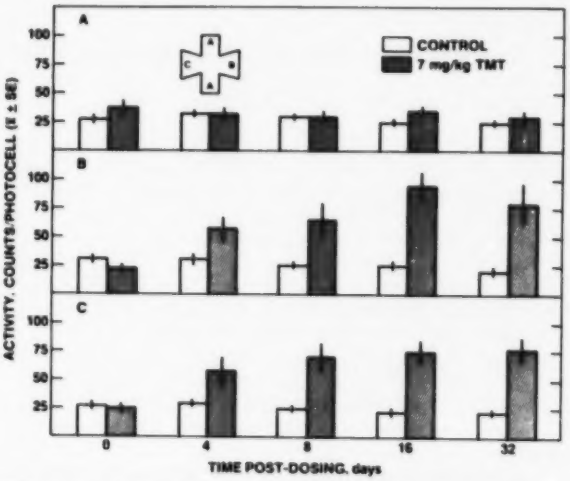

FIG. 3. Spatial distribution of activity in the figure-eight maze for control (N=10) and 7 mg/kg TMT-dosed rats (N=9) during hourly test periods. Values are mean ± SE for photocell counts in areas A, B and C of the maze.

blind alleys (Area A), the right arm of the maze (area B), and the left arm of the maze (area C). Animals exposed to 7 mg/kg TMT increased their activity in the "figure-eight" portion of the maze but not in the blind alleys. This increase in activity was symmetrically distributed in the 2 sides (B and C). For photocell counts on the right side of the maze, there was a significant effect of treatment, $F(1,68)=21.52$, $p<0.0002$, day of testing, $F(4,68)=5.08$, $p<0.0013$, and a treatment-by-day interaction, $F(4,68)=8.79$, $p<0.0001$. On day 0, the 7 mg/kg animals were slightly less active than controls, $F(1,17)=4.86$, $p<0.04$. On days 4–32, 7 mg/kg TMT animals were more active than controls, $F(1,17)=7.33$, 8.65, 30.63 and 12.49, respectively, $p<0.01$. For photocells on the

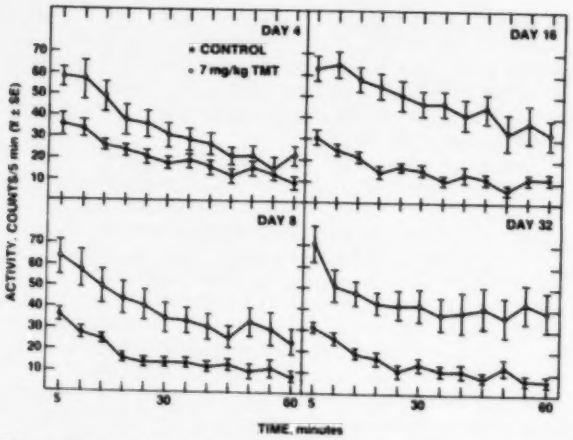

FIG. 4. Temporal distribution of activity in the figure-eight maze for control (N=10) and 7 mg/kg TMT-dosed rats (N=9) during test periods when hyperactivity was present. Values are mean±SE for 5-min intervals during hourly test periods.

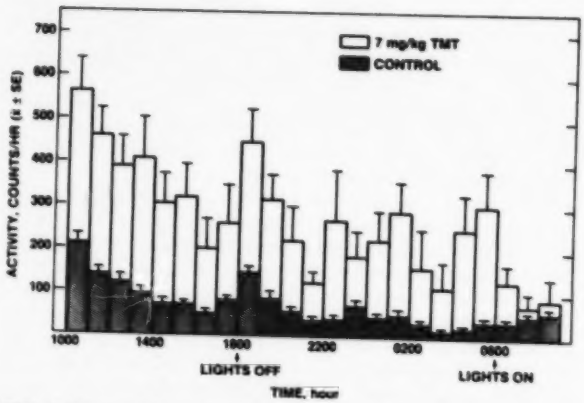

FIG. 6. Figure-eight maze activity for hourly intervals during a 23-hr test for control (N=10) and 7 mg/kg TMT-dosed rats (N=9). Values are mean±SE for photocell counts.

left side of the maze, there was a significant effect of treatment, $F(1,68)=27.55$, $p<0.0001$, day of testing, $F(4,68)=5.06$, $p<0.0001$, and a treatment-by-day interaction, $F(4,68)=9.99$, $p<0.0001$. On days 4–32, 7 mg/kg TMT animals were more active than controls, $F(1,17)=5.50$, 17.17, 33.83 and 29.64, respectively, $p<0.03$.

Data from 5-min intervals during the 1-hr test were used to determine the rate of habituation. Differences between saline controls and 7 mg/kg TMT animals were analyzed for each day of testing. There was an overall effect of day of testing, $F(4,68)=4.23$, $p<0.004$, and a treatment-by-day interaction, $F(4,68)=3.03$, $p<0.02$. Only on day 32 was the rate of habituation decreased for 7 mg/kg TMT animals, $F(1,17)=4.78$, $p<0.0431$, in comparison to controls. Figure 4 indicates the within session activity counts for controls and 7 mg/kg TMT animals for the days on which there were differences in overall activity.

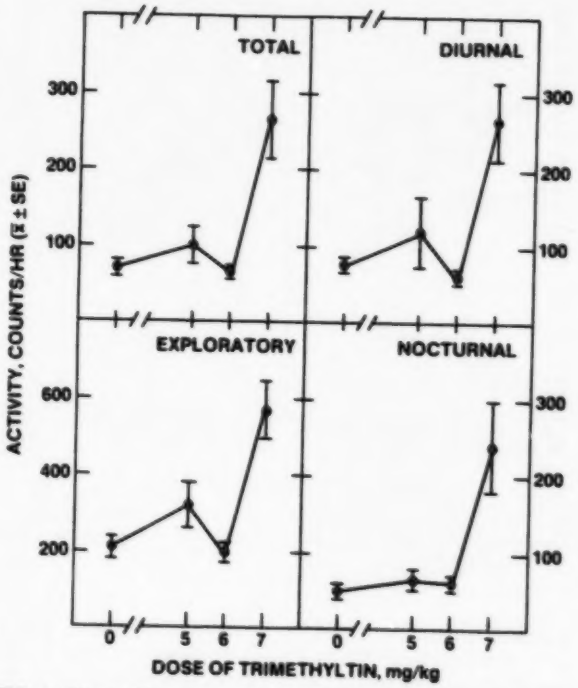

FIG. 5. Figure-eight maze activity during a 23-hr test in control and TMT-dosed rats (N=9–10/group). Values are mean±SE for photocell counts.

Maze activity for 23 hr on days 49–51 following dosing is shown in Fig. 5. Total activity was different between groups, $F(3,38)=12.98$, $p<0.0001$. Activity was increased in 7 mg/kg TMT animals in comparison to all other groups ($p<0.01$); this increased activity was observed during the 1 hr exploratory period, the diurnal period, and the nocturnal period. Activity for each hourly interval is shown in Fig. 6 to illustrate this consistent pattern of increased activity in the 7 mg/kg TMT animals relative to controls.

The length of the pyramidal cell line of the hippocampus, as shown in Fig. 7, differed as a function of treatment $F(3,29)=8.01$, $p<0.0005$. This measurement was decreased in the 5 mg/kg ($p<0.05$), 6 ($p<0.01$) and 7 mg/kg ($p<0.01$) TMT animals.

DISCUSSION

A single dose of 7 mg/kg TMT produced a persistent hyperactivity in adult rats. Hyperactivity was not present 2 hr after dosing, but was apparent by day 4 and reached asymptote on day 16. This time course parallels previously reported neuropathological changes which first appear 2 days after dosing and are maximal within 3 weeks [2]. Dyer *et al.* [7] have suggested that the neurotoxicity of TMT is similar to that of kainic acid, which also produces cell loss in the pyramidal layer of the hippocampus and hyperactivity [8,16].

Increased activity in animals with hippocampal lesions has been described as a stereotypic running rather than increased exploration [13,24]. Most studies investigating re-

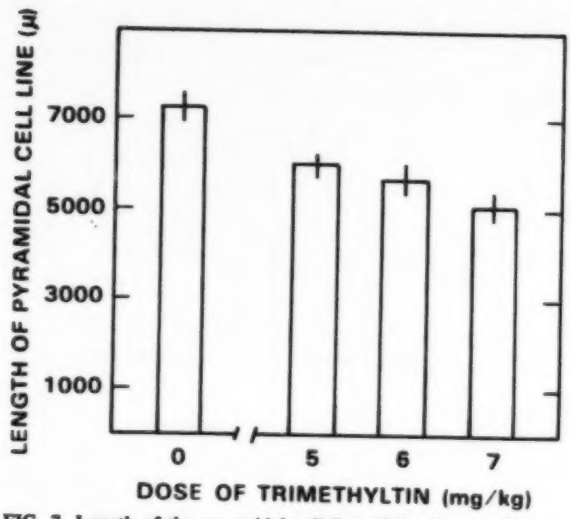

FIG. 7. Length of the pyramidal cell line of the hippocampus for control and TMT-dosed rats (N=7–9/group). Values are mean±SE.

sponse perseveration of activity have used either a T maze or a Y maze [3, 4, 15, 18, 23] which produces a decrease in spontaneous alternation in animals with hippocampal lesions. In the figure-eight maze, TMT produced a clear dissociation of activity patterns between the continuous circular portion of the maze and the blind alleys. Whereas control animals show an equal distribution of activity in all photocells, hyperactive TMT animals show a selective increase in activity in the circular alleys. This suggests that TMT produced an alteration in the pattern of activity rather than an increase in exploration. This spatial patterning is not unique. For animals with lesions in the globus pallidus, which also produce hyperactivity, increased activity is more pronounced in the continuous corridors of the maze than in the blind alleys [19].

It is controversial whether hippocampal lesions produce an increased initial activity in a specific test apparatus, or whether activity changes reflect a decrease in the rate of habituation [4, 17, 23, 24, 26]. In 7 mg/kg TMT animals initial activity was clearly increased. The rate of habituation remained relatively stable (only on day 32 did this pattern shift indicating both increased initial activity and a slower decline of activity within the test session for 7 mg/kg TMT animals) producing dramatically increased overall activity. The curves show some trend towards differences in habituation on other days which may be masked by variability. This persistent hyperactivity is in agreement with data of Swartzwelder *et al.* [25].

Several studies have indicated that hyperactivity of animals with hippocampal lesions is more pronounced during the nocturnal period [10, 11, 12]. In the present study, when activity was measured over 23 hr, the increase in activity of the 7 mg/kg group was equivalent during the diurnal and nocturnal periods. However, for this single 23-hr period, there was no circadian pattern of activity for any group. Only when animals are tested in the maze over several consecutive days does a clear circadian rhythm of activity develop [20,22]. The overlap in diurnal activity levels in the 3 hr following the nocturnal period (Fig. 6) suggests that possibly diurnal activity on subsequent days may not differ between 7 mg/kg TMT animals and controls.

The decrease in the length of the pyramidal cell line found in this study, which reflects a decreased number of pyramidal cells in area CA3 [6], confirms that definite hippocampal damage was present in TMT-dosed animals tested for locomotor activity. However, TMT produced significant morphological damage after all doses, but hyperactivity only after the highest dose. One interpretation of this discrepancy is that hippocampal morphology is a more sensitive indicator of TMT toxicity than locomotor activity, e.g., a threshold number of pyramidal cells may need to be damaged before behavior is disrupted. An alternative interpretation is that, at the highest dose of TMT (7 mg/kg), additional neuropathological damage occurs in another brain area which in turn is responsible for the hyperactivity. Although all doses of TMT produced a significant loss of pyramidal cells the degree of damage between doses was not large. Yet TMT induced hyperactivity was an all or nothing effect, i.e., it was not graded with dose, but when present, it was not a marginal effect. Thus, while systemically administered TMT clearly damages the hippocampus and clearly produces behavioral changes, caution is required in attributing this behavioral toxicity solely to disruption of hippocampal function.

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